

International Symposium on Epidermolysis Bullosa

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An international symposium on inherited epidermolysis bullosa was held at the University of North Carolina at Chapel Hill on April 25–26, 1994. All areas currently of clinical and research interest pertinent to this disease were discussed, ranging from basic epidemiologic issues to the definition of molecular defects in each of the three major types of epidermolysis

bullosa and the potential for gene therapy. A major focus of this meeting was the presentation of data collected by the National Epidermolysis Bullosa Registry. Key words: molecular biology/basement membrane/prenatal diagnosis/collagen/keratin/ultrastructure/gene therapy. J Invest Dermatol 103:839–843, 1994

Sponsored in part by a conference grant from the National Institutes of Health, the goals of this meeting were to provide a intensive program encompassing a wide variety of clinical and research topics pertinent to inherited epidermolysis bullosa, and to provide a forum in which cutting-edge findings from the laboratory could be presented alongside with focused discussions of ongoing clinical importance. A series of mini-symposia, discussion panels, and individual presentations were held. In addition, 64 posters, representing unpublished basic and clinical research, were prepared by participants and were made available for viewing and informal discussion throughout the course of this two-day meeting. Approximately 100 physicians and scientists from the United States, Canada, the United Kingdom, France, Italy, Germany, Austria, Norway, Finland, the Netherlands, Israel, Japan, and Australia attended and participated in the symposium.

SESSION I: CLINICAL AND EPIDEMIOLOGIC CONSIDERATIONS OF INHERITED EPIDERMOLYSIS BULLOSA

Mini-Symposium I: Epidemiology of Inherited Epidermolysis Bullosa Dr. Jo-David Fine (University of North Carolina at Chapel Hill) opened the symposium with an overview of the current scheme used by the National EB Registry (NEBR) for the diagnosis and subclassification of patients with inherited epidermolysis bullosa. Advantages and limitations to this classification system were discussed.

Dr. Lorraine Johnson (University of North Carolina at Chapel Hill) summarized much of the data generated to date from the NEBR. These data represented the efforts of investigators and collaborators at the four regional clinical centers (New York, Stanford, Chapel Hill, and Seattle) since inception of the project in 1986. As of December 1, 1994, comprehensive data had been compiled on 1,764 epidermolysis bullosa patients residing within the continental United States. Data were analyzed collectively as well as after stratification by NEBR clinical center, the latter done to search for possi-

ble geographic differences in the composition of enrollees or for variation in the interpretation of specific findings by different groups of investigators. About half of all patients enrolled in the project were being followed by the Chapel Hill clinical center. Approximately 25% of all enrollees were assigned new epidermolysis bullosa subtypes after comprehensive evaluation at one of the four clinical centers. One of the most significant findings noted was the rather profound overlap in many of the cutaneous findings, most notably scarring, milia, and nail dystrophy, across the four major epidermolysis bullosa groups; such overlap undoubtedly contributed to patient misclassification prior to the performance of sophisticated diagnostic laboratory tests at the NEBR. Similarly, considerable overlap was observed in the frequencies of some extracutaneous findings, suggesting potential difficulties both in prognostication and in the specificity of diagnosis, if some of these findings are used for the latter purposes. Differences were also noted in the frequency of several cutaneous and extracutaneous findings across specific epidermolysis bullosa subtypes (i.e., among Weber-Cockayne, Koebner, and Dowling-Meara variants of epidermolysis bullosa simplex). Significant interobserver variability was noted across the NEBR clinical centers when one prominent cutaneous feature (presence or absence of exuberant granulation tissue) was considered, whereas essentially little or no variability was detected when another major clinical parameter, pseudosyndactyly, was assessed. Preliminary results were also presented of lifetable analyses across major epidermolysis bullosa types and subtypes for the development of pseudosyndactyly, tracheolaryngeal stenosis or obstruction, esophageal stenosis or obstruction, and premature death.

Dr. Chirayath Suchindran (University of North Carolina at Chapel Hill), chief biostatistician for the NEBR, discussed several of the statistical techniques being employed for analysis and modeling of the extensive NEBR database. One of these techniques, classification and regression tree analysis (CART), employs a complicated, iterative, mainframe computing approach whereby patients with as yet undiagnosed types of inherited epidermolysis bullosa can be assigned with reasonable accuracy into one of seven different epidermolysis bullosa subtypes or groups on the basis of the presence or absence of one or more of a collection of selected clinical parameters. Other biostatistical techniques discussed included lifetable analysis, hazards modeling (with fixed and time-dependent covariates), and logistic regression.

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Abbreviations: NEBR, National EB Registry.

Dr. Michael Tidman (University of Edinburgh, Scotland) presented preliminary data from the Epidermolysis Bullosa Registry recently established within Scotland. As of April 1994, 223 cases had been enrolled, approximately half having some form of epidermolysis bullosa simplex. Of the dystrophic epidermolysis bullosa patients identified, three quarters were found to have autosomal dominant transmission. Overall, the prevalence of epidermolysis bullosa in Scotland was estimated to be about 45 cases per 1 million population, a figure closely approximating that reported (42/10⁶) in Norway and nearly 1.5 and 2 times that reported in Northern Ireland and Finland, respectively. Interestingly, the prevalence of epidermolysis bullosa in Scotland is slightly less than one quarter and one tenth of that noted for neurofibromatosis and cystic fibrosis, respectively, within the same population. Data were also presented on the impact of epidermolysis bullosa on employment in Scotland. Similarly, Dr. Adrian Heagerty (North Staffordshire Hospital Centre, Stoke-on-Trent, England) discussed the demographics of his own epidermolysis bullosa patient cohort, including several large informative kindreds with the mottled pigmentation variant of epidermolysis bullosa simplex and a newly identified example of cicatricial junctional epidermolysis bullosa.

Dr. Claudine Blanchet-Bardon (Hopital Saint-Louis, Paris, France) presented data on her experience with an epidermolysis bullosa patient population being followed in Paris. Although a formal disease registry does not exist in France, 257 patients, 25% of whom represent recessive dystrophic epidermolysis bullosa, have been identified via the French epidermolysis bullosa association and are being followed in a multi-specialty research clinic setting. She reported that 17 of these patients, including individuals with both recessive dystrophic epidermolysis bullosa and the Dowling-Meara variant of epidermolysis bullosa simplex, had evidence of some type of glomerulonephritis. Four patients had multiple squamous cell carcinomas.

Dr. Tobias Gedde-Dahl (University of Oslo, Norway) shared some of his extensive clinical and genetic experience with over 100 and 69 Norwegian and Swedish kindreds, respectively, who are affected by epidermolysis bullosa, including patients with one rare subtype of epidermolysis bullosa simplex, the Onga variant, which appears to exist only within a portion of Scandinavia. He noted striking differences in the incidence of specific epidermolysis bullosa subtypes within different regions of Scandinavia. Dr. I. Anton-Lamprecht (University of Heidelberg, Germany) similarly presented data on those epidermolysis bullosa cases on whom investigative studies had been performed in Heidelberg. As of April 1994, ultrastructural studies had been performed on 599 cases, representing specimens not only from Germany but also from many other European sites. Nearly equal numbers of cases of simplex, junctional, and dystrophic epidermolysis bullosa were examined, including examples of Bart's syndrome from each of these three major epidermolysis bullosa groups.

Dr. Hiroshi Shimizu (Keio University School of Medicine, Toyko, Japan) presented data on the prevalence of epidermolysis bullosa in Japan, based on a nationwide survey performed in 1983. Three-hundred ninety-three cases (epidermolysis bullosa simplex, 48.2%; junctional epidermolysis bullosa, 6.0%; dominant dystrophic epidermolysis bullosa, 18.7%; recessive dystrophic epidermolysis bullosa, 28.2%) were identified by questionnaire sampling within the departments of dermatology and pediatrics at 406 Japanese hospitals. On the basis of this survey, the prevalence for all forms of epidermolysis bullosa in Japan was estimated at 670–920 cases per 10,000 population, and the prevalences of epidermolysis bullosa simplex, junctional epidermolysis bullosa, dominant dystrophic epidermolysis bullosa, and recessive dystrophic epidermolysis bullosa were estimated to be approximately 340–470, 18–24, 130–180, and 180–250 cases per 10,000 population, respectively.

Mini-Symposium II: Carcinogenesis and Inherited Epidermolysis Bullosa Dr. Edison Liu (University of North Carolina at Chapel Hill) gave a presentation on many of the proposed mechanisms of carcinogenesis, focusing on the molecular basis of some

malignancies, particularly myelogenous leukemias resulting from ras oncogene mutations, and discussed many of the epidemiologic ramifications of such findings.

Dr. Fine next presented longitudinal data from the NEBR cohort on the prevalence and cumulative risk of epidermolysis bullosa patients developing one or more skin cancers. Data were stratified by major epidermolysis bullosa type and subtype for each of several different cancer types, including basal cell carcinoma, squamous cell carcinoma, malignant melanoma, and a group comprised of all other histologically distinct tumors. It was demonstrated that the risk of developing at least one squamous cell carcinoma in the setting of recessive dystrophic epidermolysis bullosa was 72 times that seen in NEBR patients with epidermolysis bullosa simplex. Using life-table analysis, it was further shown that squamous cell carcinomas were confined to patients with recessive dystrophic epidermolysis bullosa, that they occurred more frequently in patients with the Hallopeau-Siemens subtype, and that they became a clinically serious concern on or after about age 20, with 23.7% and 51.0% of Hallopeau-Siemens patients at risk for this tumor by ages 25 and 35, respectively. It was also reported that the cumulative risk of malignant melanoma in the Hallopeau-Siemens subtype was about 4% by age 12.

Dr. John McGrath (St. Thomas' Hospital, London, England) reported on 31 squamous cell carcinomas that arose within about a third of a 35-member cohort of recessive dystrophic epidermolysis bullosa patients. In some instances, multiple tumors arose within individual patients. Most of these tumors arose in areas of chronic scaling or ulceration and were usually histologically well differentiated, and almost all developed over bony prominences on the extremities. Worst clinical outcomes were usually associated with more poorly differentiated tumors. Dr. McGrath also reported on a recent immunohistochemical study on recessive dystrophic epidermolysis bullosa-associated squamous cell carcinomas, using an antibody specific for the p53 oncogene. Twenty-six percent of these tumors had immunohistochemical evidence of p53 expression, primarily tumors that were moderately to poorly differentiated. Although data were limited, these findings suggested that p53 expression might correlate with more aggressive biologic activity of these tumors.

Dr. Stephen Tying (University of Texas Medical Branch, Galveston, TX) discussed very recent studies on DNA and RNA extracted from a small number of squamous cell carcinomas that arose in the skin of a few Hallopeau-Siemens patients. No human papillomaviruses were detected within these tumors. However, three of the four tumors had mutations detected within the p53 oncogene, suggesting a role for p53 oncogene mutations in the evolution of these tumors. Dr. Tying also summarized a series of *in vitro* immunologic studies performed on peripheral blood cells from patients with each of the four major epidermolysis bullosa types. Marked diminution of natural killer cell activity was noted in patients with most severe disease activity, most notably those patients with the Hallopeau-Siemens subtype of recessive dystrophic epidermolysis bullosa. Similarly, the number of cells bearing markers for natural killer cells was decreased, and alterations were noted in cells bearing CD4+ and CD8+ markers. Interleukin-2 production was slightly reduced by those cells obtained from patients with junctional epidermolysis bullosa and markedly decreased in both major forms of dystrophic epidermolysis bullosa. Interleukin-1 production was similarly diminished in these epidermolysis bullosa types. *In vitro* γ -but not α -interferon production was also reduced in junctional and dystrophic epidermolysis bullosa. *In vitro* production of tumor necrosis factor α and tumor necrosis factor β was progressively diminished in junctional, dominant dystrophic, and recessive dystrophic epidermolysis bullosa patients.

Mini-Symposium III: Therapy of Inherited Epidermolysis Bullosa Dr. Eugene A. Bauer (Stanford University) gave a historical overview on the use of phenytoin and retinoids in the management of patients with recessive dystrophic epidermolysis bullosa. As he discussed, phenytoin was originally chosen for clinical trial due

to evidence of increased interstitial collagenase activity in this epidermolysis bullosa subtype, and the hypothesized inhibition of this enzyme by phenytoin, based upon early *in vitro* studies. In further support of the use of phenytoin, it was more recently demonstrated that interstitial collagenase cleaves type VII collagen, the molecular target for genetic mutation in dystrophic epidermolysis bullosa. Whereas initial open clinical studies revealed a dose-dependent decrease in blister counts with increasing phenytoin serum levels, a subsequent double-blinded, crossover, placebo-controlled, multicenter clinical trial failed to show any overall benefit. Retinoid acid was similarly considered for clinical trial in recessive dystrophic epidermolysis bullosa due to *in vitro* data, suggesting that it also partially inhibited collagenase expression in a dose-dependent manner and induced tissue inhibitor of metalloproteinases (thereby secondarily inhibiting collagenase activity). Similarly, *in vitro* studies have suggested that retinoic acid may induce expression of one or more chains of nicein/kalinin, suggesting a potential role for retinoids in the treatment of junctional epidermolysis bullosa. However, in a very small, unpublished, open trial of isotretinoin in recessive dystrophic epidermolysis bullosa, modest improvement was noted but excessive side effects prevented its further use even when very low dosages of the drug were employed. On the basis of these collective data, neither phenytoin nor any of the retinoids currently is being employed in the routine management of patients with any type of epidermolysis bullosa.

Dr. A. Griswold Bevin, Jr. (University of North Carolina at Chapel Hill) gave a personal and historical overview of the surgical management of skin cancers in patients with severe generalized recessive dystrophic epidermolysis bullosa, emphasizing the roles of both definitive and palliative surgical measures. In addition, he reported excellent results with skin grafting in over 170 reconstructive procedures performed in this unique epidermolysis bullosa subpopulation.

Dr. J. Timothy Wright (University of North Carolina at Chapel Hill) presented data from an ongoing prospective study on the nature of oral manifestations in various types of epidermolysis bullosa. He reviewed differences, as stratified across the major epidermolysis bullosa types and subtypes, in the observed frequencies of soft tissue erosions, ulceration, scarring, milia, ankyloglossia, and microstomia. Similarly, he presented data on the frequency of enamel hypoplasia and caries across all major epidermolysis bullosa types and subtypes.

Dr. Andrew Lin (Rockefeller University) discussed some 8 years' experience with the use of autologous cultured keratinocyte grafts for the treatment of non-healing wounds in patients with severe junctional and recessive dystrophic epidermolysis bullosa. Although repeated grafting was often required, lasting wound closures were achieved, thereby allowing for further reconstructive procedures to be performed, when indicated. Dr. John McGrath (St. Thomas' Hospital, London, England) then described his experience with the use of allogeneic cultured keratinocytes as skin grafts in ten patients with severe recessive dystrophic epidermolysis bullosa. When compared to non-grafted skin sites, no significant clinical benefits were obtained by the application of these allografts.

Dr. Elizabeth Fenjves (SUNY at Stony Brook) gave the keynote address on gene therapy. Both *ex vivo* (using autologous keratinocytes) and *in vivo* approaches were described. A major limitation reported with the *in vivo* technique is the current inability to specifically target the gene to a selected recipient cell type. Examples were given of specific genes that have been successfully transduced into keratinocytes in culture, of genes introduced into keratinocytes that have been measured in the systemic circulation, and of genes that appear to be at least temporarily stable with regard to their expression. Unfortunately, to date the latter phenomenon has been true only *in vitro*; *in vivo*, this gene expression is only transient. Another technical problem yet to be overcome is how to get a gene to penetrate the stratum corneum. Alternatively, it has been shown that a gene can be injected intradermally, but the latter does not allow for the targeting of specific cells. A number of specific issues pertinent to epidermolysis bullosa and gene therapy were raised. For example,

in an autosomal dominant disorder like epidermolysis bullosa simplex, it will be necessary to silence an existing gene, although it is not yet clear how this will be technically achievable. Finding a means whereby the exogenous gene can be selectively transported to its target cell will also be critical. In the case of keratinocytes, the target cell should be a stem cell, although little is yet known about this epidermal cell subpopulation. It is also unknown whether an inserted gene will be biologically active.

A panel discussion was held on other therapeutic options in epidermolysis bullosa. On the basis of the results of an ongoing study being conducted at Rockefeller University on a limited number of Hallopeau-Siemens patients, Dr. Lin recommended elective gastrostomy placement in those patients unable to take in reasonable amounts of nutrients orally, noting significant weight gains in those patients so treated. Dr. Bauer commented on a similarly positive response in gastrostomy-fed patients on both body weight and, in affected adolescents, the promotion of secondary sex changes. Dr. Anton-Lamprecht reported on the successful use of balloon-assisted dilatation of the esophagus; similar benefit with mercury-weighted dilators was noted by Dr. Fine. Dr. Bauer briefly discussed the potential benefits and limitations to the use of topical transforming growth factor β in a disease, such as dystrophic epidermolysis bullosa, that is already associated with significant dermal fibrosis. Several panel members also commented on the relative merits and risks of elective tracheostomy placement in junctional epidermolysis bullosa children having evidence of early tracheolaryngeal disease activity.

Mini-Symposium IV: Postnatal and Prenatal Diagnosis and Inherited Epidermolysis Bullosa

Dr. Karen Holbrook (University of Florida) gave a detailed presentation on the morphogenesis of normal fetal human skin and the application of transmission electron microscopy in both postnatal and prenatal diagnosis of specific epidermolysis bullosa subtypes. Over 40 such fetuses have been evaluated by her former laboratory in Seattle; no errors in diagnosis have occurred, and only a minority of these fetuses at risk for epidermolysis bullosa were shown to be affected. She also commented on the interesting morphologic finding of macrophagic-appearing cells in the amniotic fluid of mothers carrying fetuses affected with recessive dystrophic epidermolysis bullosa.

Dr. Jo-David Fine (University of North Carolina at Chapel Hill) discussed the role of immunofluorescence antigenic mapping and the findings of selected skin basement membrane-specific monoclonal antibody studies in both the postnatal and prenatal diagnosis of major epidermolysis bullosa types and subtypes. Data were presented confirming that antigenic mapping is as accurate and sensitive as transmission electron microscopy in distinguishing among simplex, junctional, and dystrophic forms of epidermolysis bullosa. Sensitivity and specificity of diagnosis were reported for monoclonal antibodies to type VII collagen (LH 7:2 epitope), kalinin/nicein (GB3), and uncein (19-DEJ-1), both when used singly and in combinations. In general, absence or diminution of type VII collagen staining was both sensitive and specific for recessive dystrophic epidermolysis bullosa, although further delineation of specific recessive dystrophic epidermolysis bullosa subtypes resulted in considerable reduction in both sensitivity and specificity. Whereas abnormal uncein staining was a highly sensitive (i.e., 100%) marker for junctional epidermolysis bullosa, the finding was not totally specific, because a minority of recessive dystrophic epidermolysis bullosa specimens also revealed similar or identical findings. Similarly, although absence of kalinin/nicein was highly specific for junctional epidermolysis bullosa, particularly of the Herlitz variant, the sensitivity of this finding was weak. However, combining the results obtained with antibodies to uncein and kalinin/nicein led to findings which were both highly sensitive and specific for junctional epidermolysis bullosa.

Dr. Sherman Elias (University of Tennessee, Memphis) gave a detailed lecture on the surgical aspects of prenatal diagnosis of inherited epidermolysis bullosa. Beginning with a historical overview of fetoscopy-directed skin biopsies, Dr. Elias then discussed more

recent sampling techniques employing smaller, flexible, biopsy forceps that are inserted into the amniotic cavity via a small bore plastic catheter and precisely guided by two-dimensional ultrasound visualization. Seventeen of the latter biopsies have now been performed in Memphis without any resultant fetal wastage; in every case, the presence or absence of inherited epidermolysis bullosa was correctly predicted on the basis of the appearance of the surgical samples so obtained. Other diagnostic prenatal sampling approaches potentially applicable to inherited epidermolysis bullosa were discussed, including amniocentesis and chorionic villus sampling; advantages and limitations of each were discussed. Finally, an overview was given on recent attempts at the prenatal diagnosis of aneuploidy using fetal nucleated erythrocytes, identified and fluorescence activated cell sorter-isolated by the presence of the CD71 transferrin receptor and glycophorin-A, which can be isolated from maternal blood samples, counterstained for the presence of fetal hemoglobin, and then studied using specific DNA probes that collectively can identify about 95% of all major chromosome anomalies.

Dr. Robin Eady (St. Thomas' Hospital, London, England) reported on over 150 prenatal diagnostic studies, some of which represented subsequent pregnancies in the same mothers, and noted that his group is now frequently obtaining fetal skin samples at earlier gestational times than in the past (i.e., 15 rather than 18 weeks). He also commented on the presence of remnants of keratinocyte plasma membranes along the bases of cleavage planes in specimens from fetuses affected with junctional epidermolysis bullosa in association with pyloric atresia. Given the normal expression of nicein/kalinin (via GB3 monoclonal antibody) in such tissue, he questioned whether these patients had a form of pseudo-junctional epidermolysis bullosa. Dr. Blanchet-Bardon reported on 86 prenatal diagnostic studies, 48 fetuses of which were at risk for the Herlitz variant of junctional epidermolysis bullosa. Similar to the experiences of other groups performing such studies, both sensitivity and specificity were 100% (i.e., no false-positive or false-negative studies), and about 30% of her cases subsequently were shown to be affected. She also reported on the successful performance of prenatal studies on chorionic villi from three mothers at risk and trophoblast sampling from an additional one, using molecular biologic probes. Dr. Shimizu commented on his experience with prenatal diagnosis in a limited number of cases at risk in Japan. Dr. Anton-Lamprecht discussed her experience with 106 prenatal diagnostic studies for epidermolysis bullosa. She reported high sensitivity and specificity for several known morphologic markers of disease (i.e., hypoplastic hemidesmosomes and junctional epidermolysis bullosa). She also commented on the morphologic reasons chorionic villi cannot be used reliably for the ultrastructural prenatal diagnosis of inherited epidermolysis bullosa at even 11 gestational weeks. Dr. Holbrook commented on some of the potential technical problems associated with the evaluation of fetal skin biopsy samples, including the frequent visualization of artifactual intraepidermal clefts associated with the surgical technique, the occasional harvesting of fetal membranes rather than skin, and several morphologic problems that may arise as a result of inadequate fixation.

Dr. Jouni Uitto (Jefferson Medical College) gave a presentation on the potential application of molecular biologic techniques to the prenatal diagnosis of selected epidermolysis bullosa types. He discussed the advantages of molecular studies over those currently employed using fetal skin samples, as well as the advantages associated with harvesting DNA from chorionic villi rather than from amniotic cells obtained via amniocentesis. He then presented examples of a few recently performed studies (i.e., evaluation of a fetus at risk for Hallopeau-Siemens recessive dystrophic epidermolysis bullosa via linkage analysis using markers for the type VII collagen gene). He also discussed the exciting potential of preimplantation diagnosis and the subsequent selection and implantation of unaffected embryos, following successful performance of *in vitro* fertilization.

SESSION II: THE BASIC SCIENCE OF INHERITED EPIDERMOLYSIS BULLOSA

Dr. Robert A. Briggaman (University of North Carolina at Chapel Hill) provided a historical perspective on those ultrastructural ab-

normalities of the dermoepidermal junction that have been associated with specific epidermolysis bullosa types and subtypes. He further commented on many of the potential problems that may arise in the diagnosis, classification, and subclassification of epidermolysis bullosa using exclusively morphologic findings as markers of disease, emphasizing both the influence of genetic heterogeneity and the likely variability of correlation between clinical phenotype and specific structural abnormalities. Drs. Tidman and Eady then reviewed and summarized their data obtained from the morphometric analysis of specific structures along the dermoepidermal junction in both normal and epidermolysis bullosa skin. Dr. Eady also presented more recent ultrastructural work employing an immunogold labeling technique, demonstrating semi-quantitative differences in the numbers of anchoring fibrils in patients with different subtypes of recessive dystrophic epidermolysis bullosa. He also discussed findings from a series of elegant morphometric studies focused on the definition of quantitative differences within the epidermis of specific structural features (i.e., keratin filaments), both in different layers of the skin and in different regional body sites.

The focus of the symposium then turned toward data derived from antibody probes directed at specific skin basement membrane proteins. Dr. Heagerty began by reviewing the collective data on staining of epidermolysis bullosa skin specimens with the anti-type VII collagen monoclonal antibody, LH 7:2. He contrasted the reported abnormal staining of LH 7:2 to two previously reported monoclonal antibody probes for dystrophic epidermolysis bullosa, KF-1 and AF1/AF2, and then discussed the parallel findings observed with LH20Sp, an anti-human sperm fibrous sheath monoclonal antibody that also binds along the human dermoepidermal junction, most likely to an epitope of type VII collagen. Finally, Dr. Heagerty discussed the ontogeny of each of these antigens in human fetal skin and then demonstrated how each associated antibody has been successfully employed in prenatal diagnosis.

Dr. Guerrino Meneguzzi (University of Nice, France) next discussed nicein/kalinin, a laminin isoform, and one of two recently recognized components of the anchoring filament. The differences in staining of skin from different junctional epidermolysis bullosa subtypes with the GB3 monoclonal antibody were summarized. He described the biochemical and molecular characterization of each of the three nicein/kalinin subunits and cited the existence of antibodies specific for each. Examples were given of altered expression (both in mRNA and actual protein production) of individual nicein/kalinin chains in some junctional epidermolysis bullosa patients. He reported that nicein/kalinin is synthesized in normal teeth by enamel-producing ameloblasts. Thus, he hypothesized that the enamel defect observed characteristically in all junctional epidermolysis bullosa patients reflects altered expression of this protein. He also reported the presence of one of the B chains of nicein and the transient detection of its A chain in the choroid plexus and spinal cord, respectively, of lower vertebrates.

Dr. Fine reported on the further characterization of uncein (19-DEJ-1), the first protein documented immunohistochemically to be an anchoring filament protein and the only protein undetectable with the dermoepidermal junction of any junctional epidermolysis bullosa skin specimen, regardless of disease subtype. As with nicein/kalinin, three uncein subunits have been detected. A product of the keratinocyte, uncein was shown to be present in cultures of keratinocyte monolayers within and on their cell membranes whereas nicein/kalinin was detectable only underneath and between these cells. Confocal microscopy studies confirmed these strikingly distinctive localization patterns for the two proteins. Furthermore, he reported that uncein, but not nicein/kalinin, co-localized with two known hemidesmosome-associated proteins, $\alpha\beta 4$ integrin and bullous pemphigoid antigen-1. He noted by both immunofluorescence and immunoprecipitation studies that uncein appeared to be trapped intracytoplasmically in cultured keratinocytes from a patient with generalized non-Herlitz junctional epidermolysis bullosa, whereas nicein/kalinin expression appeared to be unaltered.

Dr. Bauer spoke on the role of matrix metalloproteinases in epidermolysis bullosa, first reviewing *in vivo* and *in vitro* data on tissue

collagenase. Cytokines up-regulating and down-regulating matrix metalloproteinases were described. Similarly, the postulated role of *jun* and *fos* in the regulation of collagenase expression was discussed. Data were presented on the increased expression of both tissue collagenase and stromelysin-1 by recessive dystrophic epidermolysis bullosa fibroblasts, in the face of normal levels of tissue inhibitor of metalloproteinases. *In vitro* studies were presented to demonstrate that tissue collagenase does degrade type VII collagen. Finally, Dr. Bauer discussed the possibility of antisense inhibition of gene expression of interstitial collagenase.

Mini-Symposium V: Molecular Biology of Inherited Epidermolysis Bullosa

The first part of this mini-symposium, focused on epidermolysis bullosa simplex, was chaired by Dr. Elaine Fuchs (University of Chicago). Dr. Fuchs began by reviewing the structural organization of keratin filaments. She then summarized the results of a series of deletion mutagenesis experiments aimed at determining the influence of mutations within different regions of the keratin 14 molecule on the subsequent formation of a stable keratin filament network. Subsequent transgenic mouse models, using different keratin 14 gene mutations, revealed that some of these mutations resulted in phenotypes having intraepidermal blister formation and mechanical fragility, reminiscent of some epidermolysis bullosa simplex subtypes. Introduction of a mutation known to be a severe disrupter of keratin filament assembly resulted in clumping of keratin filaments similar to that observed in the Dowling-Meara variant, whereas milder gene mutations were associated with ultrastructural findings within basilar keratinocytes consistent with those observed in the Weber-Cockayne subtype of epidermolysis bullosa simplex. Immunofluorescence studies of keratinocytes from patients with the Dowling-Meara variant demonstrated a collapsed keratin network when examined with antibodies to keratins 5 and 14, and keratins extracted from these cells failed to assemble normally into filaments. Subsequently several mutations were identified in DNA from patients with Dowling-Meara and Weber-Cockayne patients within the same regions of the keratin 14 gene that were predicted by transgenic mouse experiments. Dr. Fuchs then summarized data from several other laboratories on the different sites of mutations within either keratin 5 or 14 genes in patients with each of the major epidermolysis bullosa simplex subtypes, noting that the location of these mutations correlated well with the relative severity of disease activity observed in different epidermolysis bullosa subtypes. She also noted that site-directed mutagenesis experiments in cultured keratinocytes resulted in keratin staining patterns identical to those observed in cells cultured from different epidermolysis bullosa simplex subtypes. Finally she summarized studies on epidermolytic hyperkeratosis, in which mutations recently have been mapped in the keratin 1 or 10 genes, analogous to those observed in epidermolysis bullosa simplex.

Dr. Birgitte Lane (University of Dundee, Scotland) presented data from her laboratory on keratin 5 gene mutations in epidermolysis bullosa simplex, demonstrating relative clustering of the mutations within selected regions of the gene. In the case of Weber-Cockayne epidermolysis bullosa simplex, she postulated that the structural instability of the resultant keratin molecules might be secondary to the creation of new phosphorylation sites. Dr. Irene Leigh (London Hospital Medical School, London, England) discussed mutations that she has detected within keratin 1 or 10 genes, which encode for keratins present with suprabasilar keratinocytes, in several different kindreds with epidermolytic hyperkeratosis, and summarized data from others on mutations in keratin 9, a protein confined to palms and soles, in patients with inherited palmoplantar keratoderms. She also commented on recent studies in ichthyosis bullosa of Siemens that demonstrated mutations in the keratin 2 gene. Dr. Adrian Heagerty discussed preliminary molecular studies on several kindreds with the syndrome of epidermolysis bullosa simplex associated with mottled pigmentation and punctate keratoderma. Dr. Karen Stephens (University of Washington) reported on the very unusual presence of homozygous dominant disease in a

large kindred with epidermolysis bullosa simplex of the Koebner variety. Linkage analysis and gene sequencing confirmed the mutation to reside within the keratin 5 gene. Dr. Alain Hovnanian (John Radcliffe Hospital, Oxford, England) presented an example of a unique deletion mutation in keratin 5 in a patient with the Dowling-Meara subtype of epidermolysis bullosa simplex.

The second portion of this mini-symposium was directed toward studies focused on the elucidation of genetic defects in dystrophic epidermolysis bullosa. Dr. Angela Christiano (Jefferson Medical College) began by discussing the structure of the gene for type VII collagen. She then described frameshift mutations in recessive dystrophic epidermolysis bullosa that resulted in premature termination codons and truncation of the type VII collagen. She also noted that electron microscopic findings suggest that carriers for this particular epidermolysis bullosa subtype have approximately 50% of the number of anchoring fibrils observed in genetically unaffected individuals. In contrast to the findings observed in recessive dystrophic epidermolysis bullosa, in a large Finnish dominant dystrophic epidermolysis bullosa kindred she demonstrated the presence of a heterozygous substitution mutation, corresponding to the triple helical domain of the molecule, within the type VII collagen gene. On the basis of data generated on several other informative kindreds, she postulated that the generation of truncated polypeptides of type VII collagen results in the severe Hallopeau-Siemens form of recessive dystrophic epidermolysis bullosa, whereas assembly interference within the NC-2 domain and triple helix disruption may account for disease in mitis recessive dystrophic epidermolysis bullosa and dominant dystrophic epidermolysis bullosa, respectively.

Dr. Alain Hovnanian reported on the detection of premature termination codons within the collagenous domain in a minority of patients studied who represent the Hallopeau-Siemens subtype of recessive dystrophic epidermolysis bullosa. Dr. Leena Bruckner-Tuderman (University of Münster, Germany) described a patient with recessive dystrophic epidermolysis bullosa in whom compound heterozygous mutations within the type VII collagen gene were present; one resulted in a deletion, whereas the second represented an amino acid substitution.

The final section of this mini-symposium was directed toward molecular defects and junctional epidermolysis bullosa. Dr. Tobias Gedde-Tobias described linkage studies on a series of junctional epidermolysis bullosa patients from Scandinavia; in this well-characterized study population, data suggested that some (i.e., inversa junctional epidermolysis bullosa patients) but not all junctional epidermolysis bullosa patients may be linked to mutations within the laminin $\beta 2$ gene. Dr. Jouni Uitto reported the identification of a mutation in the gene encoding for either of two of the three subunits of kalinin in two patients with junctional epidermolysis bullosa. In one kindred, having non-Herlitz disease, a homozygous in-frame exon deletion in the LAMB2 gene occurred. In a kindred with Herlitz disease, a premature termination codon resulted from a mutation within the LAMB3 gene. He also reported on four patients, collectively representing both Herlitz and non-Herlitz junctional epidermolysis bullosa, who were shown to have mutations within the LAMB2 gene of kalinin, three of which led to premature termination codons. Finally, Dr. Guerino Meneguzzi described linkage analysis studies consistent with LAMB2 gene mutation in a large informative family with Herlitz junctional epidermolysis bullosa; direct sequencing revealed a point mutation that led to premature termination codon. He also described other mutations, including exon skipping in one, within the same gene in a few other patients with Herlitz junctional epidermolysis bullosa.

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